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Effect of Actomyosin Contractility on Lamellipodial Protrusion Dynamics on a Micropatterned Substrate

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An abbreviated title: Actomyosin contractility affects lamellipodial protrusion

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ABSTRACT:

Actin polymerization-driven protrusion of the lamellipodia is a requisite initial step during actin-based cell migration, and is closely associated with attachment to the substrate. Although tremendous progress has been made in recent years towards elucidating the molecular details of focal adhesions, our understanding of the basic coordination of protrusion and adhesion, and how the two fundamental processes relate to actomyosin contractility is still inadequate. Therefore, to highlight the effect of cell-substrate interactions on the protrusive dynamics of the lamellipodia and to correlate protrusion with actomyosin activities, this study investigated the migration of fish epidermal keratocytes on fibronectin micropatterns intercalated with adhesion-suppressed gaps of varying widths. We show that insufficient adhesion associated with the gaps could limit lamellipodial protrusion such that the percentage of migrating cells decreases with an increase in gap width, and protrusion across the gaps is accompanied by ruffling. Moreover, our results suggest that up-regulating actomyosin contractility enhances the mechanical integrity of the actin cytoskeleton, leading to an increase in the width of the lamellipodia, and consequently, an increase in the percentage of cells migrating across the gaps. Thus, we demonstrate that the protrusion dynamics at the leading edge of migrating cells are functionally involved in the global mechanical regulation of actin cytoskeletal components that enable cell migration.

Key Words: Cell migration, Micropatterning, Cell adhesion, Cell protrusion, Actomyosin contractility, Cell biomechanics

1. INTRODUCTION

Cells interact with the extracellular matrix (ECM) by establishing focal adhesions (FAs) through which they sense both the physical and chemical properties of the ECM and exert traction forces[7, 8, 38]. FAs are complex structures consisting of integrin, vinculin, talin and a host of other associated molecules that link the actin cytoskeleton to the ECM[45]. While the formation of nascent FAs at the leading edge enables migrating cells to translate polymerization forces into leading edge protrusions, mature FAs are simultaneously disassembled by actomyosin-generated contractile forces, leading to retraction at the rear of the cell. Thus, the cycle of FA assembly and disassembly is closely associated with that of protrusion and retraction, and is further determined by the intricate coupling relationship between actomyosin-generated forces and traction forces developed on the ECM[4, 27, 42]. This is particularly true for rapidly migrating cells such as fish epidermal keratocytes, which exhibit remarkably well-coordinated cycles of protrusion and retraction[30].

Actin polymerization-mediated cell protrusion is closely associated with attachment to a substrate and it constitutes an important initial step during active cell migration[16]. Therefore, it is necessary to focus on the cell–ECM interactions in order to understand how the dynamics of lamellipodial protrusion are coordinated with cell adhesion. Because adhesion strength is highly dependent on the properties of the ECM[24], it is predictable that modulating the mechanical properties of the ECM would affect cell-substrate adhesion, thereby altering the

protrusion dynamics of the lamellipodia. This in turn would permit regulation of cell motility because protrusion and retraction are highly coupled events, particularly in rapidly migrating cells. For instance, it has been demonstrated that cell protrusion stalls when the retraction of the rear is inhibited[29]. In addition, the speed and morphology of migrating cells are known to depend on the rates of protrusion and retraction[11]. Thus, it is vital to understand the balance between protrusion and retraction, and how this balance regulates the overall cellular motility.

The periodic cycles of protrusion and retraction are considered to be mediated by contractile forces generated from interactions involving myosin II and the actin cytoskeleton[14]. In keratocytes, these interactions are mainly localized along the actin bundles (stress fibres: SFs) located at the back of the lamellipodia[43]. For instance, it has been demonstrated that actomyosin tension is necessary for the formation of FAs at the leading edge[9, 40] and also for the retraction of the cell rear by inducing FA disassembly[12]. Moreover, actomyosin contraction is believed to couple locally with the traction forces that a cell exerts on the ECM through FAs[22]. In addition, cycles of adhesion and retraction that mediate periodic lamellipodial contraction are suspected to synchronize with actomyosin contraction[21], but the phenomenon has not been well investigated and the exact mechanism is unknown.

The aim of this study was to investigate how the dynamics of lamellipodial protrusion are affected by properties of substrate adhesion and actomyosin tension. Specifically, this study was designed to demonstrate that the lamellipodium protrusion dynamics at the leading

edge, which locates away from the rear of the cell containing the SFs, thus generating contractile forces in migrating cells, are functionally involved in the overall mechanical regulation of complex interactions between the actin cytoskeleton and its related components[38] that enable cells to migrate.

Therefore, we investigated the migration behaviour of fish epidermal keratocytes on a glass substrate consisting of rows of regularly arranged fibronectin patterns interleaved with plasma-treated and poly-L-lysine-g-polyethylene glycol (PLL-g-PEG)-coated adhesion-suppressed gaps[15]. We reasoned that the cell protrusion dynamics could be controlled by adhesion perturbation induced by micropatterning, which is a useful tool for the control of cell adhesion by spatially restricting adhesion formation to micropatterned regions of a substrate[10, 18]. We applied micropatterning of regular rectangles with quantitatively controlled sizes and gap lengths by a microcontact printing technique to investigate the effect of actomyosin activity on the lamellipodium protrusion dynamics, particularly designed for the quantitative measurement of the lamellipodium length. In this sense, micropatterning was used as a tool to quantitatively measure the lamellipodium length of the cells in controlled cell-substrate adhesion areas based on well-developed techniques[10]. We show that insufficient adhesion associated with the adhesion-suppressed gaps could limit lamellipodial protrusion such that the percentage of migrating cells drops with an increase in the width of adhesion-suppressed gaps, and protrusion across the gaps is accompanied by ruffling.

Moreover, our results suggest that up-regulating actomyosin activity enhances the mechanical integrity of the actin cytoskeleton leading to an increase in the width of the lamellipodia, and an increase in the percentage of cells migrating across the adhesion barrier gaps.

2. MATERIALS AND METHODS

2.1 Micropatterning

This study used a protein micropatterning technique[32, 33] to fabricate glass surfaces used to investigate the impact of cell-substrate interaction on the lamellipodial protrusion dynamics during the migration of fish epidermal keratocytes. Several methods have been developed for creating micropatterns on substrate surfaces in order to control cell-substrate interactions by controlling adhesive regions[10, 39], and for simultaneously controlling both substrate rigidity and cell shape using an elastomeric micropost-arrayed surface[18]. In this study, we employed a well-developed microcontact printing technique that is increasingly being used to study a myriad of cell behaviours associated with adhesion, including migration and differentiation[23, 35, 41]. This technique involves using stamps made from polydimethyl siloxane (PDMS) to transfer a micropattern of cell adhesive proteins such as fibronectin onto a glass surface to create adhesive regions for cells.

In this study, we first developed PDMS stamps using photolithographic techniques as described previously[15]. Before stamping, plasma ashing was performed by exposing 35-mm

glass base dishes (Iwaki) to oxygen plasma for 2 min at 50 W power and 1.6 Pa pressure to remove organic matter. The stamps were then used to stamp the bottom of the dishes with fluorescently labelled fibronectin, according to the procedure described in literature[15], but with the following modifications: first, the stamps were incubated for 1 hour with a protein solution consisting of 5 µg/ml of human fibronectin (Sigma Aldrich) and 15 µg/ml of Alexa Fluor 546-labelled fibrinogen (Molecular Probes), diluted in 10 mM HEPES buffer (pH = 7.4). (2) The stamps were then quickly dried under a stream of nitrogen and placed in close contact with the bottom of the dishes for 60 sec under a light weight. (3) Next, the stamped dishes were rinsed twice with distilled water and incubated with a solution of PLL-g-PEG (1 mg/ml in 10 mM HEPES buffer) for 15 min to suppress cell adhesion on non-fibronectin areas. The PLL-g-PEG adsorbed regions of the micropattern would then become resistant to cell adhesion. (4) Finally, the dishes were rinsed twice with HEPES buffer and used in successive experiments.

The obtained micropattern consisted of rectangular patterns of fibronectin, each measuring $a \times b = 18 \times 38$ µm in size, intercalated with adhesion-suppressed gaps of varying widths: $d = 3$, 5, 7 and 9 µm. A schematic of a micropattern is shown in Fig. 1, and a fluorescent image of the obtained micropattern is shown in Fig. 1B. Cell migration occurred along the pattern rows, as shown by the arrow in Fig. 1A.

2.2 Cell Culture

To obtain fish epidermal keratocytes, scales were extracted from black tetra fish and incubated overnight in modified DMEM (Sigma Aldrich) supplemented with 15% heat-inactivated fetal bovine serum (FBS, Sigma Aldrich) and 1% antibiotics (Wako). The colony of keratocytes that had formed around the plated scales during incubation was disintegrated with 0.25% trypsin in PBS. Then the medium containing the detached cells was carefully pipetted and transferred to previously stamped glass base dish. The setup was left undisturbed for 60 min at 25°C to enable cells to re-establish and begin crawling.

2.3 Analysis of Protrusion Dynamics

In these experiments, the width of adhesion-suppressed intercalating gaps, d (Fig. 1) was varied while keeping the sizes of the rectangular fibronectin regions constant. To determine the percentage of migrating cells for a given micropattern, images of keratocytes were acquired 60 min after seeding for a period of 3 min using Zeiss Axiovert 200M microscope (Zeiss) with a low magnification lens (20×, 0.4 NA, Ph2 LD-Achroplan optics). Cells that migrated across the barrier gaps to the next fibronectin pattern within the 3 minute duration were counted and the numbers expressed as a percentage of the total cell count.

To quantify the dynamics of lamellipodial protrusion and retraction, cell behaviour was monitored on a micropattern with the widest gap (d = 9 μm, Fig. 2A). Sequential phase contrast images of 20 cells (N = 20) immobilized on this micropattern (Fig. 2B) were acquired

for 10 min at an exposure time of 0.8 s and an interval of 10 sec (n = 60 in total), using a 40×, 0.65 NA, Ph2 LD-Achroplan objective lens. After image binarization and thresholding, an edge detection function available in ImageJ (NIH) was used to obtain cell outlines with the pixel size of 300 nm and the resolution (δ) of approximately 520 nm (= 0.61λ nm/N.A.). The lengths of the lamellipodia were then determined as the instantaneous maximum distance (l) from the edge of the fibronectin pattern to the leading edge, as defined in Fig. 2C. To investigate the effect of actomyosin contractility on the lamellipodial protrusion dynamics, keratocytes were treated with 1 nM calyculin A for 15 min. Calyculin A is a widely used serine/threonine phosphatase inhibitor that elevates myosin light chain phosphorylation, leading to the activation of actomyosin contractility[26, 37].

2.4 Fluorescence Microscopy

To observe the effect of spatially controlled adhesion on the actin cytoskeletal network, keratocytes were fixed with 5% paraformaldehyde for 15 min and permeabilized with 1% Triton-X100 (Polyscience) for 10 min before incubation with Alexa-Fluor 647 phalloidin for 60 min. For the immunofluorescence of vinculin, fixed and permeabilized samples were incubated for 60 min with anti-vinculin mouse IgG primary antibody (Gene Tex) at a 100-fold dilution in PBS, followed by incubation for 60 min with Alexa Fluor 405 goat anti-mouse IgG secondary antibody (Invitrogen) at a similar dilution in PBS. Before immunostaining, the samples were blocked with bovine serum albumin (BSA) for 30 min to reduce non-specific

protein binding. Similarly, for immunolocalization of myosin II, fixed and permeabilized samples were incubated for 60 min with anti-myosin IIA non-muscle rabbit-polyclonal primary antibody (Gene Tex) at a 100-fold dilution in PBS, followed by incubation for 60 min with Alexa Fluor 488 F(ab′)2 fragment of goat anti-rabbit IgG secondary antibody (Invitrogen) at a similar dilution. Finally, microscopic imaging was performed using an Olympus FV1000 confocal microscope with a 100× oil objective lens.

3. RESULTS

3.1 Lamellipodial Extension is Affected by Actomyosin Contractility

To understand the effect of spatially controlled cell adhesion on cell protrusion and migration behaviour, we developed fibronectin micropatterns with intercalated adhesion-suppressed gaps on glass surfaces and investigated the migration of fish epidermal keratocytes on the surface. These cells are known for their rapid locomotion and simple shape, making them an ideal systems for investigating cell migration[1, 43].

First, we investigated lamellipodial protrusion on a micropattern with wide intercalated gaps (d = 9 μm), such as the one shown in Fig. 2A. As shown in Fig. 2B, cells adhered to and occupied fibronectin islands, assuming the rectangular shape of the islands in a manner suggesting that cell shape was determined by adhesion. Most cells had a large cell body that occupied most of the fibronectin pattern and a narrow rim of lamellipodia along the edge of

the pattern (Fig. 2B). These cells were mainly non-polarized and stationary (Fig. 2B); nevertheless, they exhibited active lamellipodial protrusion and retraction, accompanied by considerable ruffling because of insufficient adhesion outside the fibronectin-coated region (Fig. 2B). Because ruffling indicates adhesion instability[16], this observation concurs with earlier reports that adhesion is necessary for the formation of stable lamellipodia[3].

Next, we quantified the protrusion and retraction dynamics of the lamellipodia by determining the temporal variation in the length of the lamellipodia extended by a cell that was immobilized on a fibronectin island, as shown in Fig. 2B. For this, we measured the maximum length (l) of a lamellipodium from the leading edge to the edge of the fibronectin region, as illustrated in Fig. 2C. The maximum length was considered because it is highly probable that migration across a gap onto the next fibronectin island would begin where lamellipodial extension is greatest. Figure 2D compares the results of this analysis for untreated control cells (left: white bars) with that for cells treated with calyculin A (right: dark bars). The figure illustrates that changes in the length of the lamellipodia were because of protrusion and retraction, which is expected considering that the two events are random. In addition, the distribution shows a wide dispersion in the length of the lamellipodia for both control and treated cells, suggesting that insufficient adhesion resulted in alternating phases of active lamellipodial protrusion and retraction.

Interestingly, Fig. 2D illustrates that the distribution of the length for calyculin-treated

cells is shifted upward compared to that for untreated control cells. This demonstrates that the length of the lamellipodia increases in response to enhanced actomyosin contractility. Indeed, the mean length of the lamellipodia which was 3.4 μm before the calyculin addition, significantly increased to 4.5 μm after calyculin addition ($p < 0.01$, paired *t*-test), as shown in Fig. 2D. Thus, the result suggests that contractility plays a role in the protrusion dynamics of the lamellipodia.

3.2 Effect of Gap Length on Keratocyte Migration

Based on our analysis of lamellipodial protrusion presented in Fig. 2D, we reasoned that changing the width (d) of the adhesion-suppressed gaps would at some point prompt cell migration across the barrier gaps. We therefore varied the width of the barrier gaps while keeping the size of the rectangular patterns constant and then compared the percentage of cell migration on each micropattern for both untreated and calyculin-treated cells. As shown in Fig. 3, significantly more cells, both treated and untreated, migrated on a micropattern with $d = 3$ μm compared to a micropattern with $d = 5$ μm. However, no migration was observed on a micropattern with $d = 7$ μm, except for cells treated with calyculin. Considering that the mean length of the lamellipodia (l) for untreated cells was 3.4 μm (Fig. 2D), it is reasonable that more cells would migrate over micropatterns with $d \leq 3$ μm compared to micropatterns with $d = 5$ and 7 μm because the latter is within the extensible limit of the lamellipodia.

Cells that migrated were polarized and crescent in shape, similar to what is typically

observed for keratocytes locomoting on a glass surface[43]. Those that failed to migrate, however, exhibited intensive lamellipodial ruffling around their fixed positions. The ruffles were formed as a consequence of inefficient interaction with the substrate at the leading edges of the lamellipodia[6]. Therefore, the protrusion at the leading edge appears to be closely associated with adhesion for the stability of the lamellipodia.

Treatment of cells with calyculin to activate actomyosin contractility significantly increased cell migration, with many more cells migrating after the calyculin addition for micropatterns with d = 3 μm and d = 5 μm, as shown in Fig. 3. Indeed, percentage of cell migration increased significantly after calyculin treatment, rising from approximately 30% to a level of 50% in the case of d = 3 μm and from approximately 12% to a level of 20% in the case of d = 5 μm. Moreover, a remarkable number of calyculin-treated cells were capable of migrating even on a micropattern with d = 7 μm. Note that no migration was observed on a micropattern with this gap width in the absence of calyculin. Taken together, these results demonstrate that increased actomyosin activity enabled more cells to overcome the barrier gaps, probably by enhancing the protrusion and retraction cycles[37]. This feature is consistent with the observation that activation of actomyosin contractility induced an increase in the mean width of the lamellipodia, as previously described in Fig. 2. Another possibility is that increased actomyosin contractility could promote structural integrity of the actin filament network, thereby enhancing the mechanical integrity of the lamellipodia.

3.3 Cytoskeleton and Focal Adhesion Distributions

To investigate the cellular architecture responsible for the observations described above, we performed confocal fluorescence microscopy to visualize the actomyosin cytoskeleton and FAs for both control cells and calyculin-treated cells. Fluorescent images of the actin cytoskeleton, myosin II and vinculin for a cell immobilized on the micropattern are shown in Figs. 4A, B and C, respectively. As shown in Fig. 4A, a narrow rim of actin cytoskeleton was localized along the edge of a fibronectin pattern. Contrary to the typically observed meshwork appearance, the actin cytoskeleton of stationary cells lacked discrete meshwork lattice and organization, suggesting that adhesion is necessary for the formation of the meshwork lattice typically observed for fixed keratocytes labelled with phalloidin[37, 44].

Moreover, as immunofluorescence image of myosin II (Fig. 4B) revealed that myosin II was evenly distributed over the fibronectin micropattern, and surprisingly, it did not show appreciable colocalization with the actin cytoskeleton at the leading edge. We hypothesize that the inability of the actin cytoskeleton to form a meshwork lattice could have limited the distribution of myosin II towards the leading edge. Similarly, as shown in Fig. 4C, vinculin distribution was also limited to the fibronectin-coated area, and it showed a slight concentration at the centre and along the edge of the pattern. The characteristic distribution of vinculin within the fibronectin region demonstrates that PLL-g-PEG adsorption was effective at limiting adhesion on the barrier gaps.

In contrast, cells that migrated following the calyculin treatment appeared sharply different from their stationary counterparts, as shown in the micropattern with d = 5 μm. Unlike immobilized cells, these cells were polarized and had wide lamellipodia, which were composed of the actin cytoskeleton with a discrete meshwork appearance, as shown in Fig. 4D. Moreover, as shown in Fig. 4E, myosin II was widely distributed in the cells, illustrating that the calyculin treatment induced myosin II redistribution with actin filaments in the lamellipodia. Similar to the myosin II distribution, vinculin in treated cells was also widely distributed in the lamellipodia and the cell body region, indicating an increase in the adhesion formation. Indeed, as shown in Fig. 4F, once the lamellipodia bridged over the gap, vinculin spots could be observed even on the PLL-g-PEG adsorbed gaps.

Note that thickness of lamellipodium is very thin (150 – 200 nm)[28] and its order is comparable with that of the optical plane (confocal slice thickness). In addition, FAs in the lamellipodial region in keratocytes are widely spread without making strong spots. Therefore, FAs were not clearly observed near substrate surface in both control and treated cells in Figs. 4C and F.

4. DISCUSSION

This study investigated the effect of actomyosin contractility on the protrusive dynamics of epidermal fish keratocytes migrating on fibronectin micropatterns with

adhesion-suppressed gaps of varied widths. We observed that the barrier gaps between fibronectin patterns limited normal cell migration, such that the longer the gaps, the fewer the number of cells capable of migrating across the gaps. Indeed, as previously mentioned, keratocytes could not migrate on micropatterns with a gap width of more than 7 μm without actomyosin activation (Fig. 3), indicating that in this case, the rigidity of the actin network in the lamellipodia was insufficient to overcome ruffling and retraction that usually occur when polymerization and leading edge extension are not closely followed by attachment to the substrate[13, 31]. This scenario is depicted in Fig. 5A where the tip of the lamellipodium lifts off the substrate to form ruffles, because the lamellipodium can not firmly adhere to the PLL-g-PEG adsorbed gaps.

In contrast, when cells were treated with calyculin to activate actomyosin contractility, the resulting increase in cytoskeletal tension would predictably enhance actin network rigidity, resulting in the formation of more structurally rigid lamellipodia, as illustrated in Fig. 5B. Considering that myosin II is one of the actin filament-binding proteins[36], the stimulation of actomyosin activity could increase the stability and mechanical integrity of the lamellipodial actin network, possibly by increasing the bending rigidity of the actin meshwork. As a result, the extensible length of the lamellipodia increases (Fig. 2D), enabling cells to traverse even longer gaps (up to 7 μm, Fig. 3).

In fact, the observation that the width of the lamellipodia significantly increased

following calyculin treatment (Fig. 2D) supports the foregoing argument. This is because a wide lamellipodium can form only when the integrity and the mechanical strength of the actin meshwork that supports it are sufficient. We suggest that the increased actomyosin activity could have resulted in more filaments being pulled together by an actomyosin-dependent network contraction[44]. These filaments could then be bundled by myosin II or other crosslinking factors such as α-actinin, whose turnover activity could have increased as a consequence of actomyosin activation. Thus, increased bundling by α-actinin and myosin II can be suggested as one possible mechanism by which the mechanical integrity of the actin meshwork forming the lamellipodia could have increased.

Actomyosin interactions during fish keratocyte motility are very complex[38, 37, 43]. One of the major roles of myosin II in migrating keratocytes is the contraction at the rear of the cell within arc-shaped bundles of the SFs. In this region, active (force generating) myosin II distributes because the orientation of actin filaments is mixed (anti-parallel to each other). Contrary to this, the actin filaments in the leading edge and lamellipodial regions are oriented with their barbed ends forward (parallel to each other)[43]. Therefore, myosin II in the leading edge cannot strongly contribute to the generation of contractile force, but it may contribute by acting as a cross-linker in the network and lead to enhancement of the mechanical integrity of the actin network in the lamellipodia. Additionally, if the up-regulation of actomyosin activities leads to a higher turnover in the system [36], it may lead to more participation in the

enhancement of the mechanical integrity of the lamellipodia at the leading edge.

In the developed experimental system, the PLL-g-PEG adsorbed region was referred to as the "adhesion-suppressed" surface. Although the initial surface was treated as being non-adhesive, the surface characteristics changed over time; for example, cells started migrating onto the adhesion-suppressed surface after approximately 4 hours. This may be because of the secretion of molecules attaching to the surface, and/or unstable fibronectin dissolving into the medium. Therefore, to minimize this effect, all the observations were performed within a 2 hour time span. Because of the decreased adhesion strength, lamellipodia could not firmly adhere to the adhesion-suppressed surface, and the tips of the lamellipodia easily lifted off the substrate in the gap. However, once the lamellipodia bridged over the gap, we speculate that the lamellipodia on the adhesion-suppressed surface would have a better chance to create adhesions and have a longer time span for maturation, as vinculin distribution was observed in the gap shown in Fig. 4F.

Furthermore, we speculate that an increase in the mechanical integrity of the actin network resulting from elevated intracellular tension could have prompted the formation of rather weak FAs[25] on the gaps, resulting in increased migration across the gaps with d = 5 μm or d = 7 μm (Fig. 3). This argument is consistent with the observed presence of vinculin spots on the PLL-g-PEG-adsorbed gaps (Fig. 4E). One possible explanation for this phenomenon is that increased actomyosin activity could have induced FA formation in a tension-dependent

manner[2, 45], by strengthening the integrin-substrate bond once the lamellipodia bridged over the gap, which has been shown to depend on actomyosin tension[17, 19]. This process can be facilitated by the direct recruitment of FA molecules to adhesion-suppressed areas that would occur following increased actin filament bundling due to the actomyosin-induced network contraction[44]. Indeed, it has been suggested that a spatiotemporal feedback mechanism exists between actomyosin and FAs (cell-substrate interaction) that optimizes rapid cell migration[5, 20, 24, 38]. Similarly, myosin-dependent regulation of adhesions has also been reported in the cell-cell adherence junctions revealed by microfabricated force sensors[34]. Therefore, the formation of FAs over a gap can provide support needed to enable lamellipodial protrusion across the barrier gaps, thereby resulting in cell migration. Thus, a combination of increased cytoskeletal strength and adhesion formation as a result of actomyosin activation could be necessary for increased cell migration across adhesion barriers.

5. CONCLUSION

In this study, we have demonstrated that actomyosin contractility plays an important role in coordinating protrusion with adhesion to enable cells locomoting on substrates with spatially controlled adhesion to overcome adhesion barriers and migrate. We have shown that cells normally cannot protrude across adhesion barrier gaps with a width of more than 5 μm, but, surprisingly, they are able to do so when treated with calyculin which enhances

actomyosin contraction. We have also observed that actomyosin activation results in the formation of an actin meshwork and vinculin over adhesion-suppressed regions once the lamellipodia bridge over the gap, leading to the formation of a stable lamellipodial actin network for protrusion over the barrier gaps.

Thus, this study has demonstrated that actomyosin contractility plays important roles in the protrusion dynamics of the lamellipodia, which are crucial to overall cell migration. By extension, the results outlined here could provide insights into how cancer cells are able to migrate over substrates with different adhesion properties, as demonstrated during metastasis.

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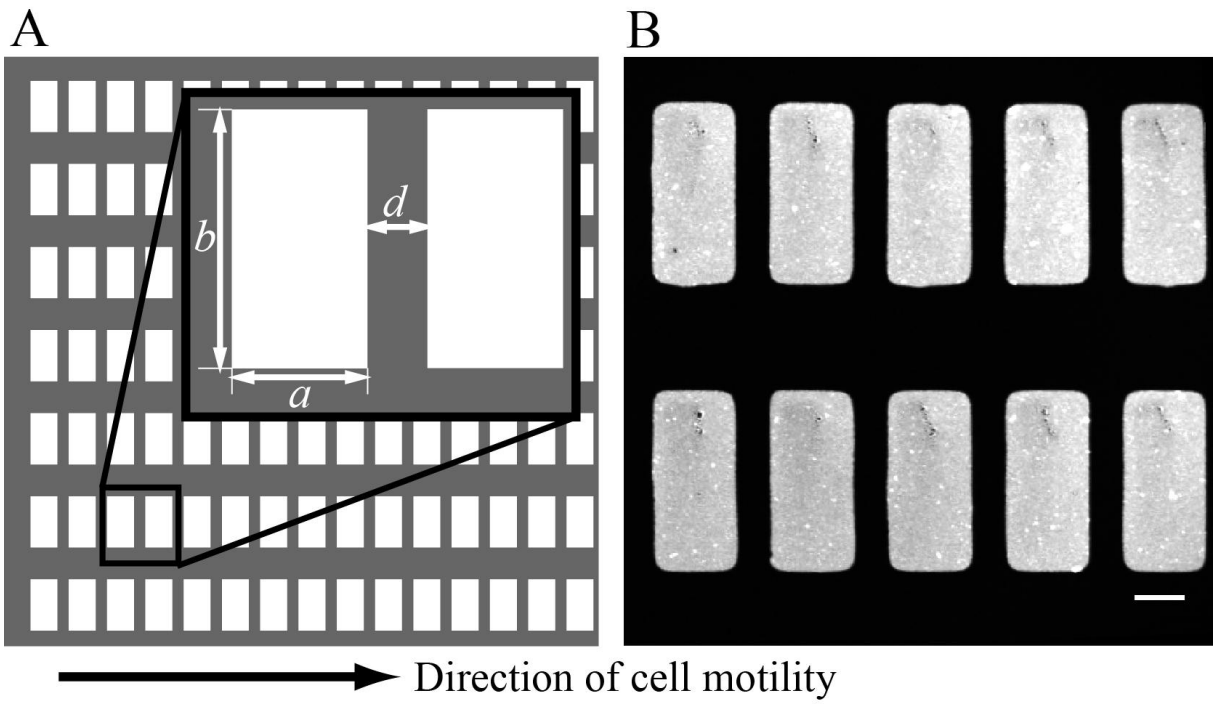

Figure 1: A micropattern consisting of rectangular patterns of fibronectin. A: Schematic illustration of a micropattern of fibronectin arrays (white small rectangles) in rows, intercalated with PLL-g-PEG-adsorbed adhesion-suppressed gaps (grey areas). The inset is a magnification showing the length (*b*) and width (*a*), of a fibronectin pattern, as well as gap width (*d*). The direction of cell migration was along the direction shown by the solid arrow. B: Fluorescent image of the micropattern labelled with Alexa Fluore 546. Scale bar is 10 μm.

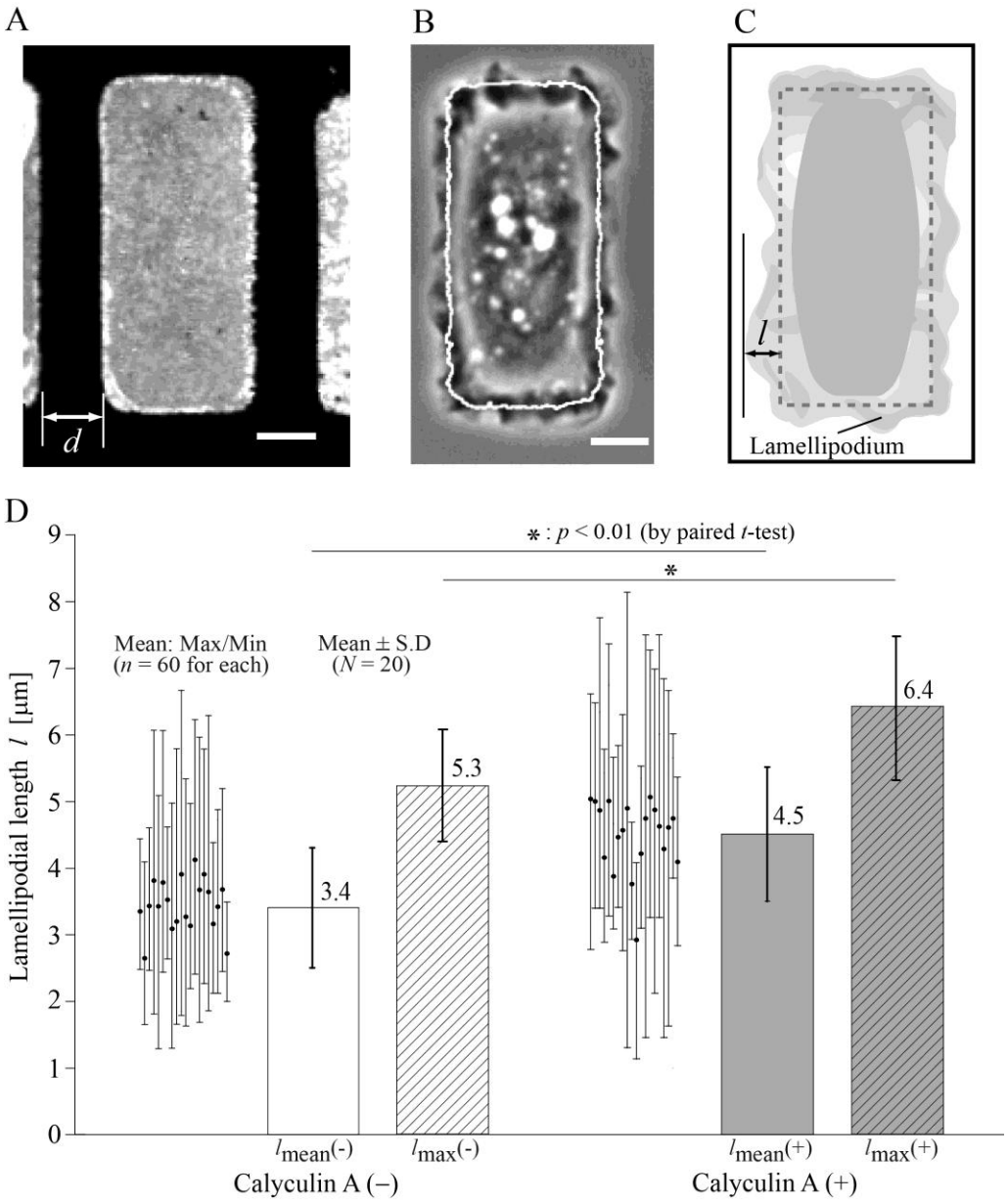

Figure 2: Cell protrusion on a micropattern. A: Fluorescent image of a fibronectin pattern on which cell migration was monitored. B: Phase contrast image of a keratocyte immobilized on the micropattern shown in A. Lamellipodial ruffling can be observed along the edge of the pattern. C: Schematic illustration of a stationary cell showing the lamellipodium and the definition of the maximum lamellipodial length, *l*. D: Distribution of *l* for both untreated control (calyculin A(-)) and calyculin-treated (Calyculin A(+)) cells immobilized on a fibronectin micropattern with a wide gap (d = 9 μm). The mean and maximum/minimum length for 60 snapshots (n = 60) for each cell (N = 20) are plotted for both calyculin-treated and untreated conditions, in which the length is shifted upward in the case of calyculin treatment, implying that elevating actomyosin contractility resulted in an overall increase in the lamellipodial length. Bar graphs highlight the increase in the mean lamellipodial length (l_{mean}) and the maximum length (l_{max}) after calyculin addition. Scale bar is 10 μm.

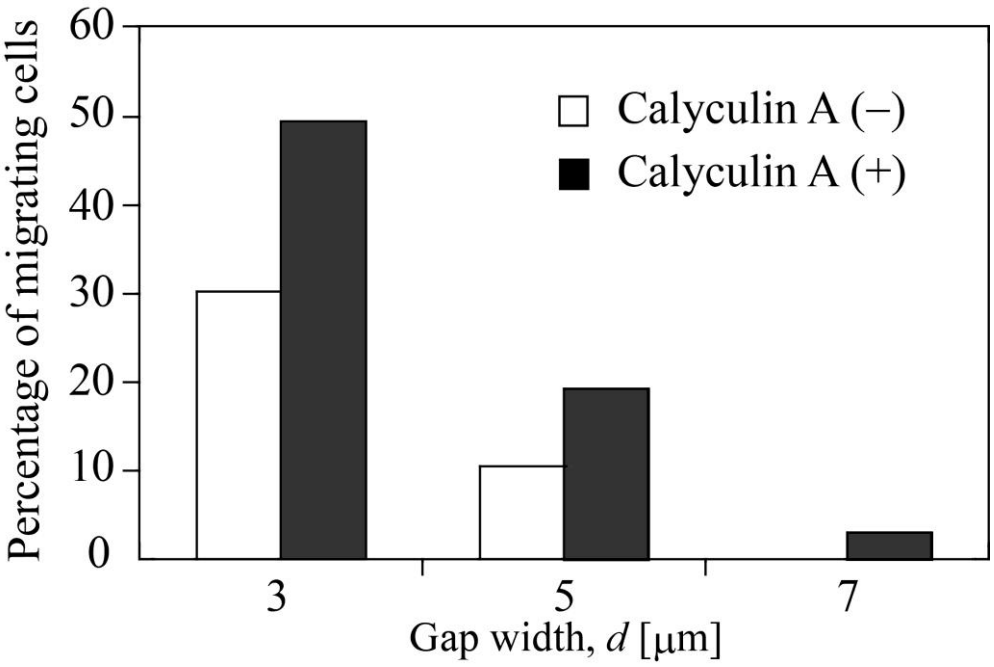

Figure 3: Effect of gap width on cell migration. Cell migration was monitored on micropatterns with a different gap width both in the presence and absence of calyculin. The percentage of cells migrating on each micropattern significantly increased in the presence of calyculin.

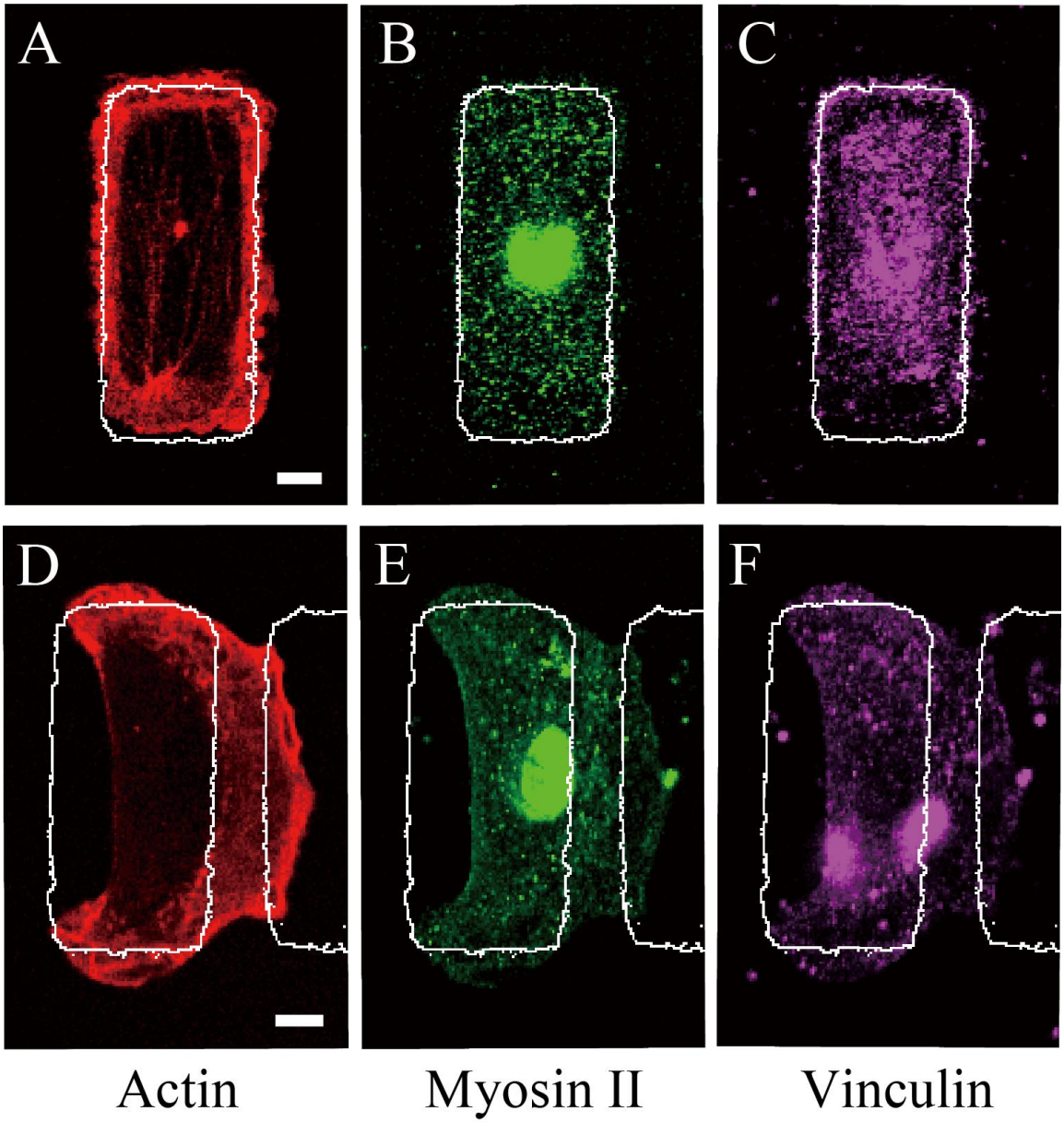

Figure 4: Fluorescence microscope images of the actomyosin cytoskeleton and FAs. A: Appearance of actin cytoskeleton for a cell immobilized on the micropattern indicated by an edge line. Actin cytoskeleton organization lacks the typically observed meshwork lattice. B, C: Immunolocalization of myosin II (B) and vinculin (C) in stationary cells. D: Actin cytoskeleton appearance in a keratocyte undergoing protrusion across adhesion-suppressed gap following actomyosin activation. E, F: Myosin II (E) and vinculin (F) distribution in a protruding cell. Scale bar is 5 μm.

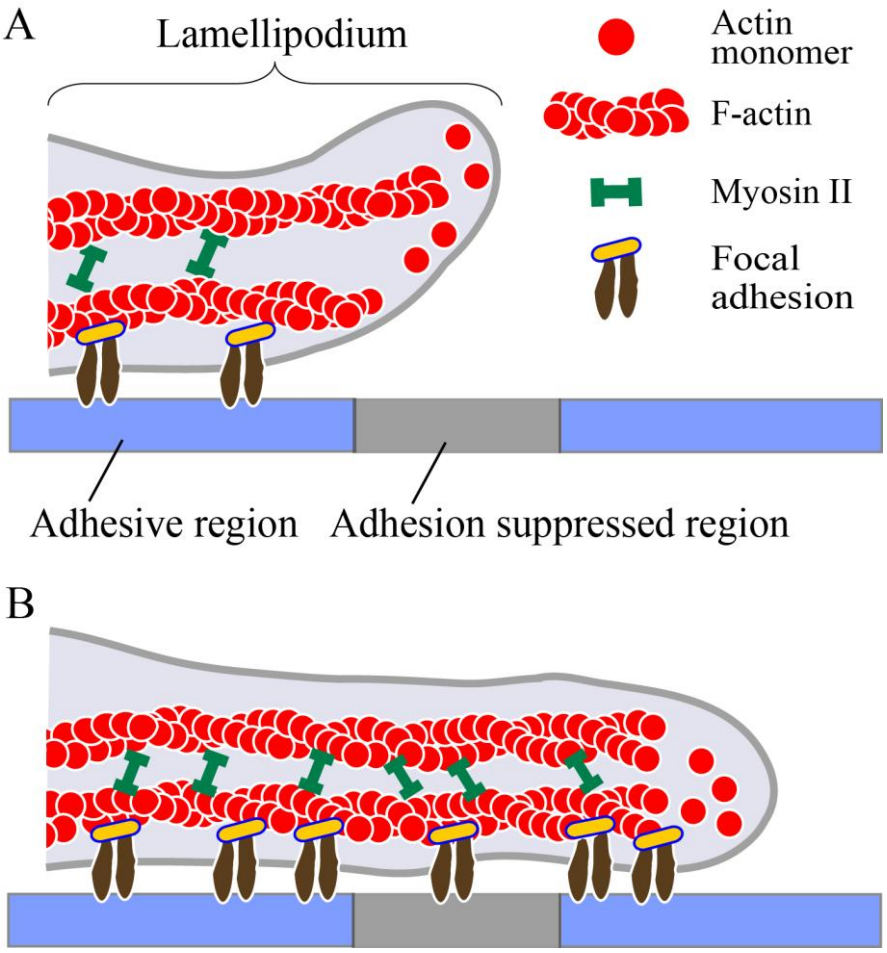

Figure 5: Schematic illustration of cell protrusion across an adhesion-suppressed gap. A: Ruffling of the lamellipodia during protrusion across a gap in the absence of calyculin. Due to its inability to firmly attach on the adhesion-suppressed gaps, ruffling occurs, resulting in the formation of an unstable lamellipodium whose actin network lacks the discrete meshwork lattice. B: Lamellipodial protrusion across a gap in the presence of calyculin. The elevated actomyosin contractility that follows calyculin treatment induces the formation of a more stable lamellipodium probably by increasing the structural integrity of the actin cytoskeleton, as well as by inducing FA formation on the gap region.